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Review Article

Epithelial polarity – Generating and integrating signals from the ECM with integrins

Aki Manninen

Biocenter Oulu, Oulu Center for Cell–Matrix Research, Faculty of Biochemistry and Molecular Medicine, University of Oulu, Aapistie 5, Oulu 90220, Finland

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ABSTRACT

Epithelial cells are important building blocks of most tissues and the corner stone of tissue architectures that allow directional transport of nutrients, ions and waste products in and out of the body. In tissues composed of millions of cells every individual cell needs to make right decisions when to differentiate, migrate, divide or die. Tight control of such fundamental cell-level processes ensures proper tissue morphogenesis, homeostasis and function. Cellular decisions are guided by biochemical and mechanical cues from their immediate microenvironment that consists of the extracellular matrix (ECM), neighboring cells and soluble factors. Generation of two distinct surfaces one facing the outside world (the apical domain) and the other contacting the neighboring cells and basal ECM (basolateral domain) is the most fundamental property of epithelial cells. The cues from the ECM are of particular importance in this process and communication between the cells and the ECM is largely mediated by transmembrane ECM receptors. Integrins constitute the largest family of such receptors binding to the ECM. Integrins have been shown to be essential for the establishment of initial polarity cues that define the position of the basal domain and thereby govern the orientation of the forming apico-basal axis. In this review I will discuss the multifaceted roles of integrins in epithelial cells with a particular focus on recent developments unveiling the specific functions of the different integrin heterodimers in regulating epithelial cell polarization and morphogenesis.

Contents

E-mail address: aki.manninen@oulu.fi
URL: http://www.oulu.fi/biocenter/groups/manninen

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Integrins in epithelial cells

Over the course of a couple of decades it has become well appreciated that integrins, a ubiquitous family of ECM receptors, are central regulators conveying microenvironmental cues to epithelial cells to regulate epithelial polarization and morphogenesis [1–6]. Large cytoskeleton-associated multiprotein complexes associated with clustered integrins not only serve to attach cells to the ECM but they are also important signaling platforms conveying multitude of signals to regulate cellular functions [7]. Importantly, integrin-dependent protein assemblies can convey information in two directions, as integrin-mediated complexes allow cells to respond to biochemical, physical and topographical cues from their microenvironment but also enable active modification (degradation, deformation, assembly of new ECM) of their immediate ECM surroundings. Given their central role in the regulation of normal development and maintenance of tissue integrity it is not surprising that integrin functions have been implicated in fibrotic diseases and cancer [8–10]. The role of integrins as adhesion receptors is known to be of critical importance but understanding the complex regulation of multiple signals triggered from integrin-complexes including crosstalk signaling between different integrin heterodimers remains a major challenge.

Integrins are the major cellular ECM receptor family that consists of 18 α- and 8 β-subunits in mammals [11,12]. At least 24 known combinations of the non-covalently linked αβ-integrin heterodimers have been reported [13]. Evolutionally, sequences homologous to parts of both α- and β-integrins are found in prokaryotes although the cell-adhesive function of integrin heterodimers probably evolved later during early metazoan development as integrins are expressed in all multicellular animals [14]. Although transcriptional regulation of integrins has been implicated in developmental and pathological processes, a highly complex post-translational regulation of integrin activity is the key factor that modulates cellular functions of integrins [15–17]. Epithelial cells express several different integrins that appear to have both unique and partially overlapping functions [18–20]. Despite extensive studies on integrin biology, several important open questions still remain. Why do cells express many different integrins that bind to same or similar ligands? How much redundancy and cross-talk there is between the different integrins? What are the molecular machineries mediating the specific functions? Excellent recent reviews have addressed the dynamics and various signaling aspects of integrin-mediated multiprotein complexes so here I will concentrate on basic aspects of integrin activation and their functions in epithelial cells [21–25]. The first part will present the key features of how integrin activation is regulated and the second part focuses on the role of integrins in epithelial cells. The current status of research is discussed with an aim to define the major open questions concerning functions of specific integrins in the regulation of epithelial cell polarity.

Integrin activation

Allosteric regulation

Regulation of integrin activation is complex and occurs at multiple levels. At a single molecule level the affinity of integrins to their ligands is highly dependent on allosteric regulation whereby conformational transitions along the entire transmembrane molecule regulate the ligand binding affinity [26,27]. Integrins were initially thought to exist in bent (inactive) and upright (active) conformations. However, later studies have shown that the correlation between integrin ligand-binding activity and conformation is much more complex and that integrins can adopt multiple intermediate conformations with different ligand-binding properties [17,27]. Integrin activation is regulated by both extracellular and intracellular factors (Fig. 1). Interactions with these factors are thought to stabilize or destabilize certain integrin conformations thereby leading to activation or inhibition of integrin ligand-binding activity [28,29].

Integrin clustering

While conformational regulation plays an important role in integrin activation at molecular level, at cellular level regulation of integrin avidity and thereby "valency" of integrin–ECM interaction sites appears to be crucial for integrin-mediated cellular functions (Fig. 2). Clustering of integrins into larger domains is required for formation of stable adhesions [30]. Several integrin clustering mechanisms have been reported. The ECM ligands of integrins are typically networks of oligomerized proteins. Clustering could thus be driven simply by binding to multivalent ligands at the sites of adhesion [31,32]. Moreover, several of the cytoplasmic integrin

effectors contain multiple protein–protein interaction domains and could therefore facilitate not only integrin activation but also function as multivalent scaffolds facilitating integrin clustering from the cytoplasmic side of the plasma membrane [33]. In particular, integrin-mediated recruitment of the actin polymerizing machinery forms actin-rich foci which anchor to the cytoplasmic domains of integrins and thereby reinforce integrin clustering [34].

Epithelial cells have often particularly prominent glycocalyx decorating the extracellular side of the plasma membrane. The glycocalyx constitutes a physical obstacle that needs to be somehow deformed or removed to allow access of integrins to their ECM ligands. Galectin-3, a multivalent lectin, is capable of forming stable lattices at the cell surface by binding to carbohydrates on both the ECM proteins and integrins [35]. Galectin-3 was found to inhibit α2β1-integrin-mediated adhesion to collagen substrate in epithelial cells [36]. Recently it was suggested that the ECM ligands at sites of initial integrin-mediated ECM contacts where glycocalyx is already compressed are closer to plasma membrane and can thus facilitate further clustering of integrins [37,38]. Clustering is possibly also enhanced by the ability of β1-integrin transmembrane domains to homo-oligomerize [39,40]. The natural propensity of lipids to form distinct domains within cellular membranes is likely to play an active role in the integrin clustering process [41]. Such coalescence of small and highly dynamic lipid rafts into larger domains has been reported to drive clustering of α5β1 and αVβ3-integrins in model membranes [42]. In addition, clustered integrin-mediated adhesions coincide with highly ordered lipids domains [43]. Integrins may also themselves actively contribute to formation of clustered lipid rafts at the cell surface [44]. Certain members of the tetraspanin protein family have been implicated as regulators of integrin recruitment into specific more ordered lipid domains [45]. Integrins can be palmitoylated themselves but the multiple palmitoylation sites in tetraspanins likely contribute to their lipid domain-organizing properties [45–47].

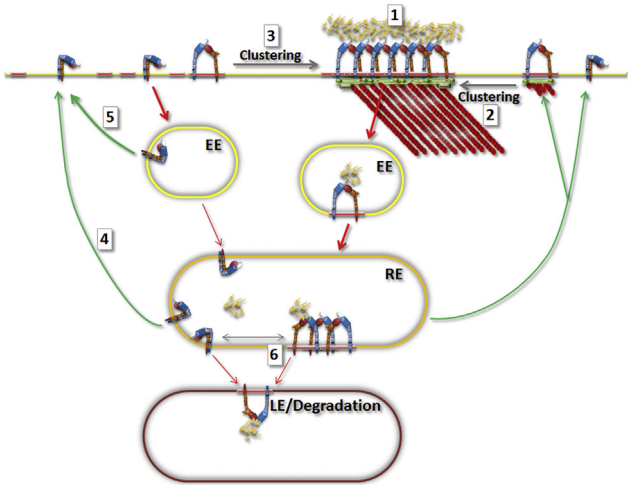

Fig. 2 – Integrin surface availability and avidity as regulatory mechanisms to control activation of integrin signaling. (1) Oligomeric ECM ligands provide multiple adjacent binding sites for integrins and thereby contribute to integrin clustering. (2) Similarly, formation of actin rich foci can reinforce integrin clustering. (3) Activated integrins may preferentially partition in lipid raft domains and coalescence of lipid rafts into bigger platforms can trigger integrin clustering. The surface availability of integrins is continuously regulated by both (4) slow (via recycling endosomes (RE)) and (5) fast (via early endosomes (EE)) endocytic recycling loops. (6) Endocytic trafficking routes of integrins can also be regulated by ligand binding.

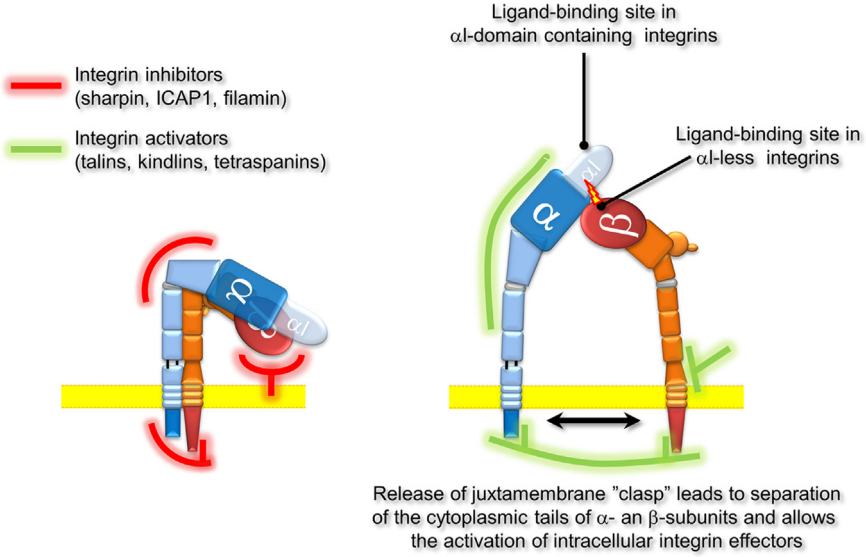

Fig. 1 – Allosteric regulation of integrin activation. Integrins exist in a bent "off-state" and extended "on-state". These conformations can be stabilized by a variety of integrin-binding proteins and/or lipids (schematically shown by green and red lines). Several inhibitory or activating integrin antibodies act by specifically binding to and stabilizing inactive or active conformations, respectively [164]. Red and green linear structures are schematic and do not necessarily correspond to actual binding sites of the listed or any other regulatory proteins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Integrin trafficking

Surface availability of integrins contributes to integrin avidity for its extracellular ligands. Integrin trafficking is thus critically important for the regulation of integrin functions in cells. Integrins are constantly being internalized from and recycled back to the cell surface [48,49]. Integrins can undergo fast recycling through early endosomes (EE) or slow recycling through perinuclear recycling endosomal (RE) compartment [50]. The endosomal route of integrins can also be modulated depending on whether integrin is in ligated or unligated state [51,52]. This membrane traffic is under tight regulation that orchestrates polarized cellular functions required for example for directed cell motility. Importantly, integrins also modulate cellular signaling by regulating trafficking of other membrane proteins, such as growth factor receptors [22,23]. While some of this regulation appears to be based on specific direct interactions between integrins and the target proteins, integrins could also have more global cellular effects via their ability to control lipid raft content at the plasma membrane [53].

Cell–matrix interactions and the regulation of epithelial cell polarization

Understanding the molecular mechanism regulating epithelial cell polarity at molecular level in single cells is a challenging task. While data from animal models provide important insight into functions of integrins at organism level including their involvement in diseases and helping to identify potential integrin effectors, the *in vivo* phenotypes are often too complex to readily assist in understanding the specific roles of integrins at cellular level [12,28]. A vast majority of current molecular knowledge of epithelial cell polarization therefore comes from simple cell-based systems, mainly established cell lines but also primary cell cultures [54,55]. *In vivo* cells are generally embedded within a three dimensional (3D) matrix made up of polymerized ECM molecules and neighboring cells and thus they need to generate the apical "free" surface de novo. Epithelial 3D cell culture models which mimic the *in vivo* organization of epithelial cells have been especially important in revealing the molecular machineries involved in epithelial polarization and morphogenesis [55–58].

Epithelial cells have an intrinsic program that drives generation of three surface domains (1) apical (free) surface, (2) lateral domain interacting with neighboring cells via adherens junctions (AJs) and tight junctions (TJs) and (3) basal surface contacting the ECM [59]. Schematically, epithelial cell polarization in typical *in vitro* 3D-conditions could be divided into distinct steps or processes (Fig. 3). It should be noted, however, that these dynamic events are highly interconnected and thus the hierarchy and sequence of some of the events remain unclear.

(1) Individual contact naïve cells have no surface polarity and they express basolateral and apical (red) surface proteins along the entire surface domain [60] (see Fig. 3).
(2) Adhesion onto the surrounding stroma (typically rich in fibrillar collagen – depicted as red-striped green bars) to form initial cell–matrix contact sites [1,4,6,61].
(3) Maturation of these cell–ECM adhesions triggers removal of apical membrane proteins and lipids via endocytosis from the membrane domain contacting the ECM [62,63].
(4) Together with the forming cell–cell junctions, these cell–matrix adhesions serve as signposts to orchestrate polarized secretion and/or assembly of laminins forming the epithelia-specific basement membrane (BM) [61,64].
(5) Spatial cues from cell–ECM and cell–cell interfaces also contribute to asymmetric targeting of intracellular polarity protein complexes (the Crumbs-complex, the Par-complex, and the Scribble-complex) [65,66].
(6) The polarity complexes together with basally assembled BM provide positional information allowing reinforcement of the polarized alignment of intracellular cytoskeletal networks [66] and thereby polarized organization of the membrane trafficking networks.
(7) Polarized membrane trafficking contributes to the generation of apical lumen de novo [67,68].
(8) The polarized organization of the organoid (cyst, acinus) is maintained via regulated orientation of mitotic spindles according to signals emanating from cell–matrix interactions [4,69,70], cell–cell junctions [71] and apical membrane domain [72].

Role of integrins in the regulation of epithelial cell polarization

Integrin-mediated signaling enables cells to probe and respond to the ECM (outside-in signaling) and to assemble and/or modify the existing ECM (inside-out signaling). *In vivo* epithelialization often coincides with synthesis and assembly of a laminin-rich basement

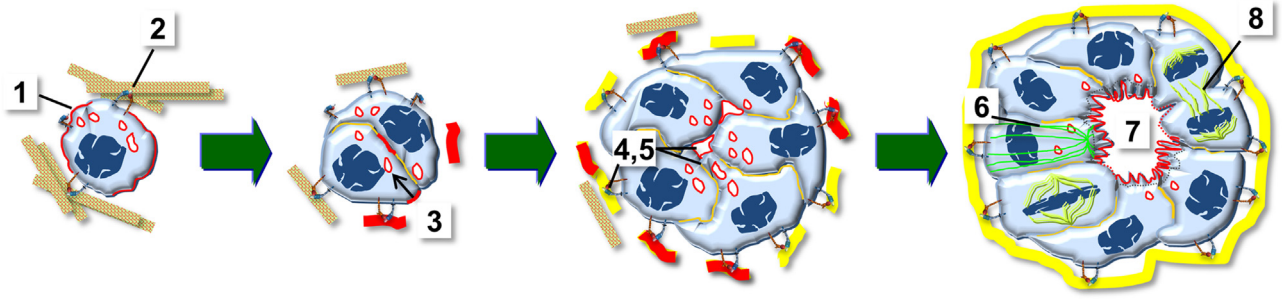

Fig. 3 – Polarization of epithelial cells in 3D organotypic cyst culture (see main text for details).

membrane (BM) that is largely produced by epithelial cells themselves [73]. Consequently, epithelial cells in various tissues are almost invariably underlined by BM. Prior to epithelial differentiation mesenchymal cells are unpolarized and often surrounded by a collagen-rich stromal matrix. In *in vitro* cultures such as described in Fig. 3, epithelial cells must secrete their own ECM and assemble a laminin-rich BM to establish the basal cue that determines the forming apico-basal polarity axis. *In vivo*, epithelial cells are accompanied by various stromal cell types of which fibroblasts are a particularly rich source of ECM [74,75]. Indeed, a reciprocal communication between epithelial and stromal tissues is of critical importance not only for normal development of most tissues but also in pathological conditions such as cancer [76,77]. Stromal fibroblasts secrete and assemble stromal ECM in a process that depends on both integrins and non-integrin ECM receptors and thereby synergistically create a common microenvironment with the adjacent epithelial cells [78–80]. Epithelial cells also contain many non-integrin receptors for both stromal ECM and BM which certainly contribute to cellular responses but it appears that integrin-mediated linkage between extracellular matrix and intracellular cytoskeleton is crucial for establishing the initial polarity cues as will be discussed below. Fig. 4

Establishing the basal cue with integrins

Establishment of cell–ECM contacts with stromal collagen-rich matrix via α2β1-integrins

When embedded into collagen gels non-transformed kidney and mammary epithelial cells adhere via α2β1-integrins [4,6,81–84]. Although some epithelial cells express significant levels of collagen-binding α1β1-integrins they appear to play only a minor role [85,86]. The expression levels of two more recently discovered collagen receptor integrins, α10β1 and α11β1, are usually low in normal epithelial cells but they could contribute to pathological conditions within the epithelium [87,88]. In individual cells and

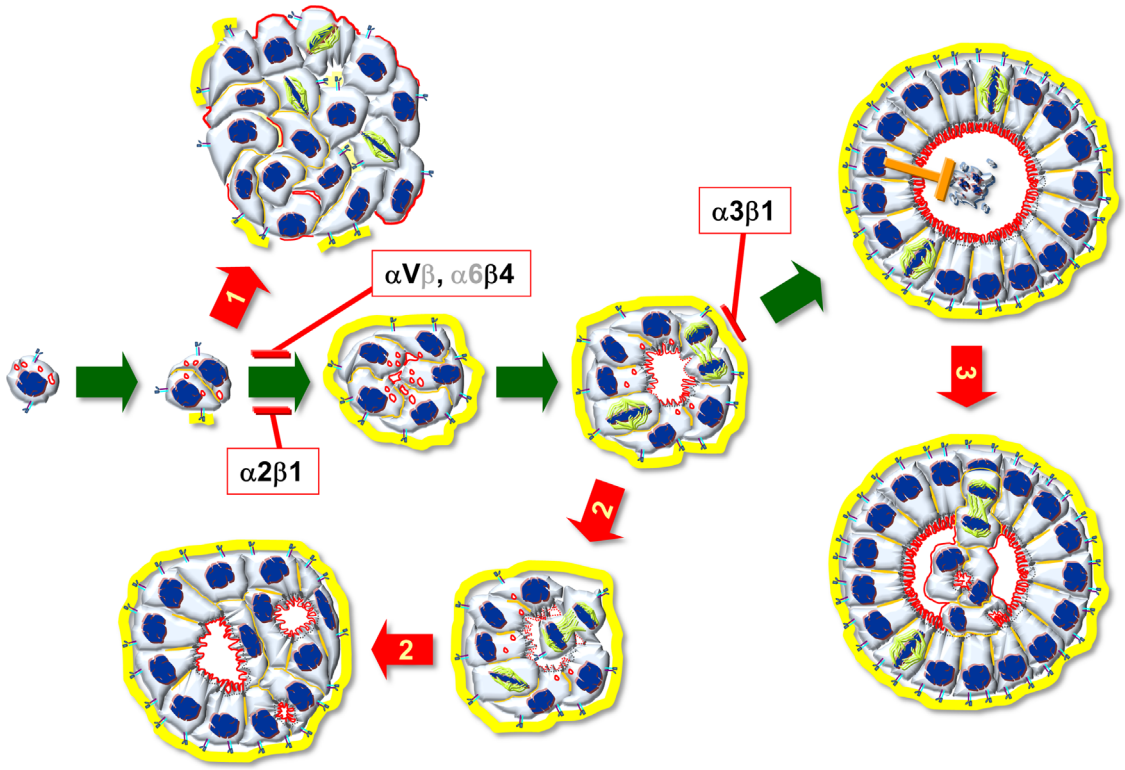

Fig. 4 – Summary of the phenotypes of integrin-depleted epithelial cells in 3D organotypic cyst culture system. When normal epithelial cells are embedded in 3D collagen gel, cells proliferate to form cell–cell contacts and establish a basal cue by binding to ECM and produce their own BM (yellow). These cues direct polarized apical membrane trafficking (red vesicles) towards the center of the growing cell cluster. Cell–cell contacts mature and form subapical tight junctions that physically segregate apical (red) and basolateral membrane domains. Cells in polarized cysts continue to proliferate and the polarized cellular organization is maintained due to controlled perpendicular orientation of mitotic spindles in relation to the apico-basal axis. In cells where α2β1- or α6β4-integrin function was inhibited, cells failed to properly assemble BM leading to growth of unpolarized cell mass (Red arrow 1). Inhibition of α3β1-integrin function led to a failure to orient mitotic spindles within the polarized epithelium leading to partially polarized structures with dysplastic features (Red arrows 2). Normal cells undergo anoikis when trapped inside the apical lumen. Mutant cells resistant to anoikis could continue proliferation thereby disrupting epithelial morphogenesis (Red arrow 3). Such phenotype was not seen in integrin-deficient "normal" MDCK cells but it has been noted in other systems and/or upon overexpression of oncogenes [5]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

small non-polarized cell clusters in 3D collagen gels both basolateral and apical membrane proteins (such as free surface/glycocalyx marker podocalyxin) (gp135; [89,90]) are found at the basal plasma membrane facing the ECM substrate (Fig. 3; [60,91,92]). Formation of α2β1-integrin-mediated adhesion complexes initiate the basal cue leading to specific removal of apical membrane components from this domain [4,62]. What controls the specific endocytosis of apical proteins is not known but the apical cargo appears to end up in Rab11a/Rab8a-positive endosomes [68]. α2β1-integrins bind collagens and laminins but the specific requirement of α2β1-integrin in collagen gels is likely due to apparent lack of other collagen-binding integrins in normal epithelial cells [4,82,92]. However, there is also evidence that lack of α2β1-integrins cannot be functionally fully rescued by α1β1-integrins [93]. Thus, α2β1-integrins are required to demarcate the site for deposition of BM which in turn orients the apico-basal axis [61,73]. Curiously, α2-integrin knockout mice are viable and show relatively moderate phenotypes in platelet activation, mammary ductal branching and glomerular function [94–96]. In the glomerulus α2-integrin is mainly expressed by podocytes. Although α2-integrin knockout glomeruli have abnormal bulges in their glomerular basement membranes and display fibrotic features this only leads to mild proteinuria presumably due to compensatory functions of other collagen receptors [94]. Importantly, because the different cell types synergistically create a proper glomerular microenvironment it is possible that adaptive responses by the other glomerular cell types, for example by inducing ECM deposition, could be crucial for maintaining the kidney function in α2-integrin deficient mice.

Mechanical maturation of α2β1-integrin-mediated focal adhesions – the regulatory role of αV-integrins

In the Madin–Darby canine kidney (MDCK) epithelial cell model α2β1-integrins mediated the initial adhesion to collagen substrate [4,97]. It was noted, however, that spreading of the substrate-bound MDCK cells required also αV-integrins although the latter did not directly contribute to collagen adhesion [97]. αV-integrin knockdown MDCK cells also displayed a moderate cystogenesis defects in collagen gels (unpublished observation). Functional integrin signaling depends on the formation of micrometer-size mature focal adhesions in which several integrin heterodimers cluster to form a robust link between the ECM and cellular cytoskeletal networks. Most integrins share many key downstream effectors activated upon cell adhesion, such as FAK and Src and Rho GTPases [98]. However, the fine-tuning of these signals seems to be mediated by specific integrin subunits which thus have distinct or only partially redundant functions [99,100]. It was found that αV-integrins contribute to the regulation of RhoA/ROCK/myosin II-dependent mechanical maturation of α2β1-integrin mediated focal adhesions [7,97]. A strikingly similar scenario was reported in a fibroblast study where αVβ3-integrins were implicated as critical mediators of mechanosensitive growth of α5β1-integrin containing focal adhesions [101]. Interestingly, quantitative proteomics approaches have revealed a striking correlation between mechanosensitive maturation of focal adhesions and recruitment of LIM-domain containing proteins such as vasp, zyxin or migfilin to focal adhesions [102]. Finally, a comprehensive analysis of the proteomes of αV- and β1-integrin-mediated adhesions in fibroblasts suggested that α5β1-integrins link to RhoA/ROCK/myosin II pathway involved in force generation whereas the αV-class integrins recruited GEF-H1/RhoA/mDia cascade required for growth of adhesions in response to force [103]. Because all integrins are thought be able to respond to mechanical forces and can also associate with other mechanoresponsive molecules, the seemingly specific role for αV-integrins in regulating mechanosensitive maturation of focal adhesions is surprising [104]. One possible explanation may come from the complex regulation of the intracellular contractile forces generated by myosin II [105]. The balance of myosin II-generated intracellular forces linked to cadherins at cell–cell junctions and to integrins at cell–ECM connections define epithelial cell morphology and regulate morphogenic events in epithelial tissues [106]. Regulation of the relative activities of RhoA/ROCK/myosin II-pathway and Rac1-pathway is of critical importance in defining both apico-basal polarity as well as front-rear polarity [107,108] (see also Control of the cell division axis within epithelium). It would be tempting to speculate that in epithelial cells α2β1- and αV-integrins recruit distinct set of effectors which synergistically fine-tune the activation/in-activation of Rho GTPases or the different myosin II isoforms [106]. However, whereas αVβ3 and α5β1 co-cluster in fibroblasts on fibronectin substrate, αV-integrins do not appear to co-cluster with α2β1-integrins in epithelial cells seeded on collagen [97,101]. Therefore, the molecular mechanisms of the αV-integrin-mediated regulation of α2β1-integrins in epithelial cells remain to be characterized.

Synergistic functions of α2β1- and β4-integrins in regulating basal laminin assembly

Laminin is the most critical component of the BM and assembly of the laminin network is a prerequisite for polarized epithelial morphogenesis [1,61,109]. Although laminin can self-assemble in solution the laminin assembly *in vivo* and in cellular systems is a cell-driven process that involves concentration of secreted (or exogenous) laminin at the cell surface [110]. When mammary or kidney epithelial cells are embedded into 3D gels made of laminin-rich basement membrane extract (BME) they form polarized cysts independently of α2β1-integrins [4,6]. High concentration of exogenous laminin might allow laminin self-assembly thereby precluding the need for α2β1-integrin dependent targeting of biosynthetic laminin transport to basal cell surface. In addition to α2β1-integrins, β4-integrins were also found to be required for the establishment of basal cue in collagen-embedded MDCK cells [4]. β4-subunit pairs with α6-subunit to form α6β4-integrin that is a laminin receptor [111,112]. In contrast, both α2-integrin knockdown (Itgα2-KD) and Itgβ4-KD MDCK cells formed normal polarized cysts in laminin-rich BME gels [4]. Abrogation of cystogenesis in BME gels required inhibition of both α2β1- and α6β4-integrin functions indicating that either one is sufficient for determining apico-basal axis but integrin-mediated signals are nevertheless crucial also in the presence of exogenous laminin [4].

How could α2β1- and α6β4-integrins synergistically regulate laminin assembly in the MDCK cell model? Laminin assembly is driven by associations between the laminin N-terminal (LN) domains located in the ends of the short arms of cross-shaped laminin trimers [110]. The dimensions of laminin (~35 nm short arms and ~75 nm long arm [113]) and BMs thickness observed *in vivo* (~20–100 nm) suggest that laminin is unlikely to stand in upright orientation at the cell surface but rather lies horizontally

in assembled BMs such that all of the LN domains are accessible to cell surface receptors including integrins that extend ~20 nm from the membrane. This is also supported by the finding that the LN domain of the alpha chains is a ligand for α2β1- and α1β1-integrins [114,115]. An intriguing possibility is that capture of the LG-domains by β4-integrins and binding of α2β1-integrins to the LN domain orient the laminin molecules at the cell surface and thereby synergistically facilitates laminin assembly when the amount of laminin is limited. Interestingly, a study utilizing GD25 cells with defined integrin expression found that while α6β4-integrins could mediate cell adhesion to truncated laminin isoform LN-332 cell spreading on this substrate required β1-integrins [116]. In contrast, α6β4-integrins were sufficient for both adhesion and spreading on network-forming laminins LN-511 and LN-521 [116]. Thus it is possible that β1- and β4-integrins are required to efficiently coordinate the BM assembly that involves transition from a non-network forming LN-332 into network-forming LN-511 [117]. A puzzling finding in the study by Myllymaki et al. was that while β4-integrin-KDs show a cystogenesis defect in collagen gels α6-subunit knockdown MDCK cells did not replicate this phenotype although β4-integrin is expected to pair only with α6-subunit to form a functional heterodimer [4,13]. Whether this discrepancy is due to formation of α6β1-integrin heterodimer in Itgβ4-KD cells subunit, or if β4-subunits have α6-subunit-independent functions remains to be addressed in further studies. It should be noted that similar to laminin, the deposition and assembly of hensin, an ECM molecule associated with terminal differentiation of epithelial cells, was shown to depend on αVβ1-integrins whereas the signals from the hensin matrix to drive phenotypic conversion required α6β4-integrins [118].

Integrins and signals of polarity

The role of small GTPases Rac1 and RhoA and microtubule targeting to integrin-mediated adhesions at the cell–ECM interface

Laminin assembly is critically important yet not sufficient for apico-basal polarization as indicated by the inability of MDCK cells to establish basal cue even in the presence of exogenous LN-111 when both β1- and β4-integrins are inhibited [4]. Which are the signals triggered upon formation of α2β1- and/or α6β4-integrin-mediated adhesions to establish the polarity cue at the basal membrane? Small GTPases are among the key effectors implicated in cellular response upon adhesion. In particular, Rac activation regulates actin polymerization in cellular protrusions including lamellipodium where integrin-mediated adhesions are initially formed [119]. Integrin clustering triggers activation of Rac1 in a manner that depends on the cytoplasmic tails of the integrin β-subunits [120–122]. Indeed, dominant-negative mutant of small GTPase Rac1 prevents removal of basally expressed podocalyxin in collagen I gel embedded MDCK cysts [61]. Organized microtubular network is a hallmark of polarized epithelial cell and in line with this model both integrin-mediated adhesions and Rac1 activation have been associated with recruitment of microtubule plus ends to FAs [62,123]. However, the overall picture of the regulatory mechanisms at FAs is complicated by several seemingly contrasting findings. A recent study reported that integrin-mediated signaling via ILK, but not Rac1, is critical for polarized morphogenesis of mammary epithelial cells *in vivo* and *in vitro* [62]. In addition, anchorage of microtubules at FAs is thought to facilitate FA disassembly in a manner dependent on the endocytic machinery and RhoA activation [124,125]. Whether or not Rac1 activation is required for basal cue it could help to inhibit RhoA to prevent FA disassembly at the nascent integrin-mediated adhesions [121,126]. Remarkably, inhibition of the RhoA/ROCK1/myosin signaling module was shown to be sufficient to restore basal cue even when DN-Rac1 was expressed or when β1-integrins were blocked using function-blocking antibodies [107]. Growing microtubule plus-ends themselves generate a positive feedback loop with Rac1 activation that maintains dynamic Rac1 activation at the newly-formed FAs [127,128]. Interference with the microtubular dynamics leads to disrupted apico-basal polarity [62]. Streuli and coworkers found that ILK links integrins with a microtubule plus-end binding EB1 and thereby anchors dynamic MT plus-ends to orient the cellular polarity axis [62]. It should be mentioned that Akhtar et al. used BM-extract gel as a 3D environment in their studies while O'Brien et al. studied MDCK cells in collagen I gels [61,62]. Addition of exogenous LN-111 to collagen I grown cysts expressing DN-Rac1 rescued the basal endocytosis of podocalyxin stressing the importance of laminin as a basal cue [61]. Although inhibition of the RhoA/ROCK/myosin-signaling axis correlates with basal laminin assembly in MDCK cells, in mouse salivary gland epithelium inhibition of ROCK perturbed BM assembly [107,129]. Thus it seems that different epithelial cell types may vary in their requirements for small GTPases in morphogenetic processes. It is also evident that while a lot of research has focused on Rac1, RhoA and Cdc42 the small Rho GTPases family consists of 20 members in mammals only some of which have been studied in epithelial cells [119,130].

Phosphoinositides – generation of basolateral and apical membrane identities

Although the exact molecular hierarchy of the signals mediating the basal cue remains unresolved elegant work by Mostov and colleagues demonstrated that phosphoinositides (PIs), $PI(4,5)P_2$ and $PI(3,4,5)P_3$, play a crucial role in defining the apical and basal membrane domain identity, respectively [68,131]. Active maintenance of PI asymmetry within cellular membranes has long been known to be a key mechanism regulating compartmentalization of cellular membranes, cytoskeleton and organelles [132–134]. In polarizing epithelium phosphatase and tensin homolog on chromosome 10 (PTEN) is recruited to apical membrane where it metabolizes $PI(3,4,5)P_3$ into $PI(4,5)P_2$ [135]. PIs facilitate targeting of numerous cytoplasmic proteins to the membranes by interacting with multiple effector domains such as plekstrin-homology (PH)-domains, phosphotyrosine binding (PTB)-domains or band 4.1, ezrin, radixin, moesin (FERM)-domains [132]. PTEN-driven $PI(4,5)P_2$ enrichment at apical domain facilitated recruitment of annexin 2 and active Cdc42 which in turn were required for activation of the aPKC/Par6 complex at the apical membrane [135]. Curiously, efficient maturation of integrin-mediated adhesions was reported to depend on the ability of talin to recruit Type I phosphatidylinositol 4-phosphate 5-kinase γ (PIPKIγ) [136]. PIPKIγ targeting to FAs was found to be necessary for local production of $PI(4,5)P_2$ and coupling of adhesions to actin

cytoskeleton. However, as mentioned above, PI(4,5)P_2 is considered to be a marker for apical membrane domain [68]. A possible explanation is that local production of PI(4,5)P_2 is transient and while it allows positive feedback signaling to promote actin polymerization and strengthen the FA-actin cytoskeleton linkage it is rapidly metabolized to other PI species at the basolateral membrane to prevent apical identity at the basal side. In support with a transient role for PI(4,5)P_2 synthesis at forming focal adhesions it was shown that binding of PIPKIγ with talin actually seems to compete with talin binding to the cytoplasmic tail of β1-integrins [136].

Maintaining epithelial polarity

Control of the cell division axis within epithelium

Integrin signaling also activates PI3K which can phosphorylate PI(4,5)P_2 to produce PI(3,4,5)P_3 [137,138]. In fact, integrin-mediated activation of PI3K leading to enrichment of PI(3,4,5)P_3 at mid-section of the cell cortex has been reported to be critical for proper alignment of mitotic spindles in dividing cells [69]. Spindle alignment directs the cell division axis and is thus essential for proper orchestration of epithelial monolayer and/or multilayer organization. In the cyst assay defects in spindle polarity lead to formation of abnormal multilumen cysts. In addition to PI(3,4,5)P_3, Cdc42 together with several of its GEFs and polarity complex proteins (discussed below) cooperate to position and anchor spindle microtubules to the cell cortex according to extracellular cues [70,139–143]. Multiple mutually reinforcing extracellular cues from apical [72], lateral [71,144] and basal [4,69] domains ensure tight regulation of the cell division axis. Interestingly, silencing the expression of α3-integrins (but not α2- or α6-integrins) in MDCK cysts embedded in BME gels resulted in deregulation of endogenous Cdc42 activity, mistargeting of active Cdc42 from apical to basolateral membrane domain and misalignment of mitotic spindles [4]. Similar findings have been made also using mammary epithelial cells and integrin function-blocking antibodies [6]. In addition to basal cell–ECM adhesions α3-integrins are abundant at lateral membranes and α3-integrin-deficient kidney epithelial cells show defective cell–cell junctions [145,146]. Further support for the specific important role for α3-integrins in epithelial morphogenesis comes from α3-integrin knockout mice which have defects in their kidneys, lungs and epidermis [147,148]. In migrating cells α3β1-integrins appear to regulate spatial activation of Rac1 and localization of the Par complex (discussed below) via interactions between a Rac-GEF Tiam and talin [149]. Cdc42 activation is required upstream of Rac1 in migrating cells but whether α3β1-integrins are directly regulating Cdc42 activation remains to be studied [150].

Sorting and maintaining polarized membranes

While integrin-mediated targeting of microtubule minus ends to the basal side is important to establish the basal domain equally critical for epithelial polarization is polarized trafficking of apical cargo to the forming pre-apical patch or apical membrane initiation side (AMIS) [60,68,151,152]. The bulk of apical and basolateral membrane cargo is sorted and packaged into distinct transport vesicles and tubules at the trans-Golgi network (TGN) [153]. In non-polarized epithelial cells apical and basolateral cargo are both targeted to adherent basal surface whereas upon cell polarization adherens junctions (AJs) serve as lateral guideposts for microtubule targeting [154]. These tracks apparently carry initially both cargoes to lateral domain to drive AMIS formation which in turn will define the site of tight junction (TJ) assembly [151,155]. In polarized cells only basolateral cargo is targeted to lateral membrane underneath the TJs whereas apical cargo appears to fuse directly with the apical domain [154,156]. The three polarity complexes; Crumbs (Crumbs/PATJ/PALS), Par (Par3/Par6/aPKC/Cdc42) and Scribble (DLG, LGL, SCRIB), come into play within these processes [155]. Targeting of the Par complex to AMIS triggers a positive feedback signal that eventually via directed exocytosis of Rab8a/Rab11a-positive apical carriers leads to the formation of pre-apical patch (PAP) [68]. Crumbs complex defines the apical "free" domain together with specific Par complex components Par6/aPKC/Cdc42 [157]. TJs serve as diffusion barriers to prevent mixing of apical and basolateral membrane proteins and lipids but polarity complexes are required to actively maintain and regulate polarized cellular organization. Asymmetric enrichment of PIs is likely an important initial event contributing to spatially polarized recruitment of the polarity complexes [155]. The segregation of apical and basolateral domains is further driven by mutual exclusion of apical (Crumbs/Par) and basal (Scribble) complexes at their respective domains. The kinase activity of aPKC stimulated by GTP-bound active Cdc42 and their recruitment to the forming apical domain by the Crumbs complex is central for removing components of the Scribble/Lgl complex from this domain. Conversely, Par1 serine–threonine kinase phosphorylates components of apical polarity complexes thereby preventing their localization to the basolateral domain [155]. The epithelial polarity program constitutes a fascinating intrinsic feedback loop whose regulation has been extensively reviewed elsewhere [151,155].

Little evidence exists for direct interactions between integrins and the polarity complex proteins. Active Cdc42-GTP is a crucial component of the Par complex and required for the maintenance of apical domain identity [135]. Integrins can activate Cdc42 in adhering cells but so far the majority of reports support a role for Cdc42 GEFs localized at tight junctions, centrosomes, or along the apical membrane trafficking pathway [139,140,158,159]. As noted above, silencing of α3-integrin in epithelial cells enhances Cdc42 activity and leads basal mislocalization of Cdc42 and abnormal cystogenesis [4]. However, the molecular mechanisms underlying these effects require more detailed studies. Integrins could regulate cellular localization of polarity complexes by modulating synthesis of the different PI species but whether more direct mechanisms linking integrins with the functions of polarity complex proteins exists is currently unknown. One notable finding in the above-mentioned studies is that, at least in fully polarized epithelium, both apical and basolateral polarity complex components are largely excluded from the cell–ECM interface. One exception is Par1b whose targeting to basal membrane has been shown to promote BM assembly in the outer columnar cells of the salivary gland epithelium [129]. In mammary epithelial cells Par1b-mediated phosphorylation of RNF41 was required upstream of basal laminin deposition [160]. This effect seems to be context and/or cell type-specific because in MDCK cells Par1b overexpression interferes with BM assembly while in inner polymorphic cells of the salivary glands inhibition of Par1b

activity via integrin-mediated activation of ROCK1 was found to limit laminin deposition [129,161].

Conclusions

The roles of laminin-rich BM and integrin-mediated signaling as critical regulators of epithelial cell polarization have been clear for several years but only recently the specific functions of individual integrin heterodimers have become more apparent. An important focus for current and future studies is to delineate the composition of protein complexes associated with specific integrins in different conditions [103,162,163]. Such molecular approaches initially require simplified cellular model systems before going into more complex *in vivo* models. However, given the dramatic effects on the cells by their immediate biochemical and biomechanical microenvironment it will be necessary to design the experimental settings such that they mimic the appropriate natural cellular microenvironments in the best possible way. Molecular details underlying the specific functions of different integrin heterodimers will help to develop new strategies to treat diseases such as fibrosis and cancer which affect epithelial tissues.

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